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# Biodegradation of volatile hydrocarbons in five surface waters tested as composed mixtures in the µg/L range

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## 1. Introduction

Biodegradation is an important removal process for many chemicals that are released to the environment. Models have been developed for prediction of biodegradability for chemicals where experimental data do not exist [1]. However, high quality experimental data are required for the development and verification of high quality models. In the development of the BioHCwin model, data from field studies and grab sample studies in which environmentally relevant concentrations were tested were preferred, however inclusion of screening test data was in some cases necessary due to a lack of more relevant data [1]. For possible oil components, biodegradation tests, at low concentrations and using a mixture of hydrocarbons (allowing co-metabolism) are more relevant to environmental conditions than single component studies, since these are released to the environment as mixtures.

Many factors affect the biodegradation of hydrocarbons, and some of these can be controlled in biodegradation tests [2]. However the microbial population is not controlled, and therefore a source of variability in biodegradation tests as well as in the environment. In this study the variability of biodegradation in five different surface waters in Denmark with different characteristics regarding pre-exposure to petroleum hydrocarbons was investigated. The study was conducted with a composed mixture of 9 hydrocarbons that were all within a narrow carbon number range (9-12 C) but diverse in molecular structure.

## 2. Materials and methods

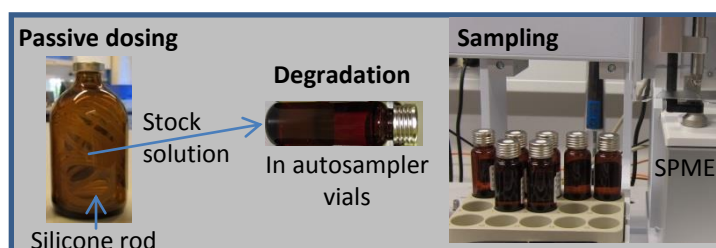
Test chemicals included n-Decane, Tetralin, Biphenyl, trans-Decalin, Bicyclohexyl, 1,2,4-trimethylbenzene, Naphthalene (Sigma-Aldrich), 2,3-Dimethylheptane, 1,3,5-Trimethylcyclohexane (TCI) at a purity of ≥ 98%.

The five different surface water grab samples which were used as inoculum in the biodegradation test are presented in Table 1 as well as background measurements used to characterise the samples.

Site	Discharges*	pH	Temperature (°C)	NVOC** (mg/L)	HPC*** (CFU/mL)
Maglesø (lake)	No	8.0	11.2	7.4	$1.4 \cdot 10^3$
Lyngby Lake	SW and WW	8.0	9.9	7.7	$4.6 \cdot 10^3$
Fønstrup brook	No	8.0	8.5	10.3	$1.2 \cdot 10^4$
Gadevangs brook	WW	7.7	10.1	6.1	$1.5 \cdot 10^4$
Harrestrup stream	SW	7.8	9.3	5.7	$8.0 \cdot 10^4$

**Table 1: Surface water samples used as inoculum in biodegradation test, \*SW=Upstream stormwater discharges WW=upstream wastewater treatment plant effluent, \*\*NVOC=non-volatile organic carbon, \*\*\*HPC=heterotrophic plate count**

The test method was based on OECD 309 but adapted for hydrophobic and volatile test chemicals (see Figure 1). Stock solution of ~ 1/100 of the solubility for each chemical was prepared by partitioning based dosing from a loaded silicone polymer rod [3]. 20 mL test systems were then prepared using 13.5 mL of the surface water inoculum and 1.5 mL stock solution. A similar number of abiotic controls were prepared using MilliQ water. Test systems were incubated at 20 °C for a maximum of 28 days on a roller and at fixed time intervals three replicate test systems and abiotic controls were analyzed on GC-MS using fully automated Head Space Solid Phase Micro Extraction (HS-SPME). At each time point the primary biodegradation was evaluated by the relative response in the test system compared to the abiotic control.



**Figure 1: Three phases of the biodegradation test method.**

### 3. Results and discussion

The general order of degradation in the five surface waters was n-Decane > Bicyclohexyl > 1,2,4-trimethylbenzene ~ Biphenyl ~ Naphthalene ~ Tetralin > 2,3-Dimethylheptane > Decalin > 1,3,5-Trimethylcyclohexane. The test system half-lives of a representative selection of the test chemicals are listed in Table 2. Water phase half-lives can be calculated taking partitioning in the test system into account. Generally the half-lives were similar in four of the surface water samples and lower than predicted using the BioHCwin model. However, 1,3,5-Trimethylcyclohexane had a predicted half-life of 3.5 days but was only degraded in one sample, Harrestrup, after all other test chemicals were degraded. In the sample from the clean lake Maglesø half of the half-lives were higher and half lower than predicted in BioHCwin.

Half-lives (days)	n-Decane	Bicyclohexyl	Biphenyl	2,3-Dimethylheptane	Decalin
Maglesø (lake)	1.0	1.5	9	9	28
Lyngby Lake	0.1	0.4	0.2	2	6
Fønstrup brook	0.2	2	0.9	1.3	5
Gadevangsrenden	0.5	2	0.9	0.8	7
Harrestrup stream	0.07	1.2	0.3	0.2	2
BioHCwin prediction [4]	9	27	31	8	68

Table 2: Half-lives (days) for biodegradation of a representative selection of test chemicals in the test systems.

Two distinct degradation phases were observed in all surface water samples. n-Decane and Bicyclohexyl were degraded quickly after a short or no lag phase (< 2 days). The four aromatic test chemicals, 2,3-Dimethylheptane and Decalin were degraded simultaneously after a lag phase which increased with decreasing number of colony forming units in inoculum from pre-exposed sites (1-3 days for Harrestrup to 3-5 days for Lyngby lake). Longer lag-phases were observed in inoculum from the relatively unpolluted sites (3-6 and 5-8 days for Maglesø and Fønstrup brook). Generally the degradation was fast for the four aromatic test chemicals and 2,3-Dimethylheptane and slower for Decalin. Degradation curves for Bicyclohexyl and Decalin are shown in Figure 2.

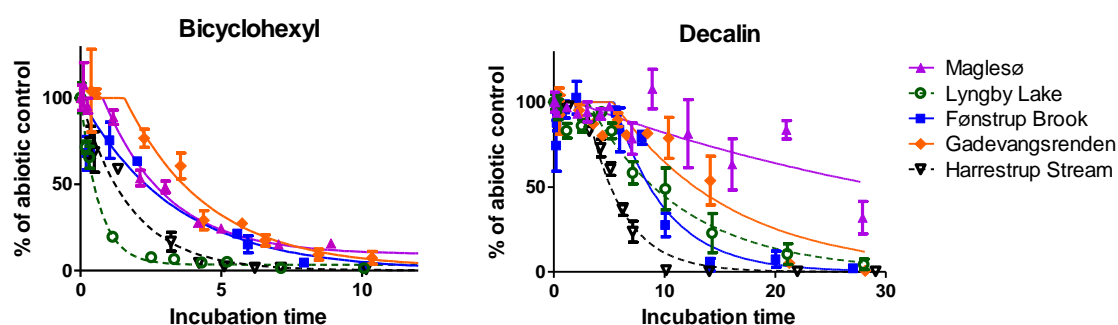


Figure 2: Degradation curves for Bicyclohexyl and Decalin. Average and Standard Error of Mean (SEM) shown for each time point, as well as fit of 'plateau followed by one phase decay'.

### 4. Conclusions

Biodegradation tests using natural surface water as inoculum revealed that for four Danish surface waters, half-lives for 9 hydrocarbons in the tests were lower than predicted using the BioHCwin prediction model, whereas degradation in the fifth sample from a clean lake was closer to the prediction in BioHCwin.

### 5. References

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